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Colloidal complexation of zein hydrolysate with tannic acid: Constructing peptides-based nanoemulsions for alga oil delivery

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ABSTRACT

Colloidal complexation of zein hydrolysate (ZH) with tannic acid (TA) and the possibility of using the ZH-TA complex to construct a nanoemulsion system for alga oil delivery were investigated. Turbidity and fluorescence titration measurements demonstrated the complexation of ZH with TA, which leads to the formation of ZH-TA complex. Isothermal titration calorimetry further confirmed the complexation between ZH and TA was driven by the non-covalent interaction (e.g. hydrophobic interaction and hydrogen bonding). The emulsifying activity of ZH was considerably improved after the complexation with TA, which was responsible for the fabrication of alga oil nanoemulsions (d = 120 nm). The emulsions stabilized by the ZH-TA complex showed a remarkable physical stability and high alga oil encapsulation efficiency. More importantly, the complexation of ZH with TA also endowed ZH-TA complex with high antioxidative properties. The alga oil nanoemulsions stabilized by ZH-TA complex showed an increased oxidative stability with reduced lipid hydroperoxides and volatile hexanal compared with that of stabilized by ZH alone. This study demonstrated that ZH-TA complex could be employed as an emulsifier in constructing a physical stable nanoemulsion delivery system, and opens up the possibility of utilizing peptides–polyphenol complex as efficient emulsifiers to improve the oxidative stability of O/W nanoemulsions.

1. Introduction

Nanoemulsions are very fine emulsions made of lipid droplets of nanometric size (d < 200 nm), produced by low-energy (e.g. spontaneous emulsification) or high-energy methods (e.g. high pressure homogenization) with the presence of adequate emulsifiers (Mcclements, 2012). Nanoemulsions have a number of potential benefits for certain applications. They allow for efficient and controlled delivery, encapsulation, enhanced solubility, bioavailability and bioaccessibility of various lipophilic food components (Adjonu, Doran, Torley, & Agboola, 2014). However, a large amount of surfactant (20%–30% based on the oil phase, wt%) must be used in the formulations to stabilize nanoemulsions, which hinders the application of nanoemulsions due to toxicological concerns during long-term exposure (Wei, Tan, Tian, Chen, & Wu, 2011). Therefore, there is an increasing interest within the food, beverage and pharmaceutical industries in utilizing edible nanoemulsions to encapsulate, protect and deliver lipophilic functional components, such as oil-soluble flavors, vitamins, nutraceuticals, and so on (Mcclements, 2011).

Although generally considered amphiphilic, not all proteins (particularly true for plant proteins, e.g. corn protein and gluten) possess sufficient surface activity to stabilize the emulsions due to their poor water solubility (Wan, Guo, & Yang, 2015). The stabilization of emulsions with the protein hydrolysate deriving from the enzymolysis of native food proteins (e.g. corn glutelin, skipjack roe protein, egg white protein and cod protein) have been well investigated in recent years (Chen, Chi, Zhao, & Xu, 2012; Farvin, Andersen, Nielsen, Jacobsen, Jakobsen, Johansson, et al., 2014; Intarasirisawat, Benjakul, Visessanguan, & Wu, 2014; Zheng, Wang, Liu, Sun, Zheng, Wang, et al., 2015). It has been reported that the protein hydrolysate have a higher rate of diffusion to the O/W interface and cover a larger area of the interface than native proteins (O'Regan & Mulvihill, 2010). Recently, whey protein hydrolysate (treated by chymotrypsin with a hydrolysis degree of 10%) stabilized nanoemulsions (d = 287.9–192.5 nm) has been reported (Adjonu et al., 2014). But, numerous evidences also show that

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protein hydrolysate alone, especially the hydrolysate with a high hydrolysis degree, cannot supply the required interfacial rigidity to stabilize the emulsions for long-term storage (Cheng, Xiong, & Chen, 2010; Jamdar et al., 2010).

Zein is one of only few known proline-rich and hydrophobic proteins. The presences of high amount of hydrophobic amino acids make zein practically insoluble in water, which is responsible for its poor functionality (e.g. emulsification) in aqueous solutions compared with other proteins (e.g. soybean protein concentrates or whey protein isolate). Protease has been widely applied in the modification of zein, and the resulted hydrolysate were characterized by prominent antioxidative (Kong & Xiong, 2006) and angiotensin converting enzyme (ACE) inhibitory activities (Ren, Ma, Mao, & Zhou, 2014). However, the evidences regarding to the interfacial activity and emulsifying properties of ZH has not been reported so far. In fact, we have recently found the amphiphilic characteristic of ZH, and the curcumin nanocomplexes can be acquired by the complexation between ZH and curcumin (Wang, Wang, Yang, Guo, & Lin, 2015). Hence, it is worth trying to utilize ZH to stabilize the O/W emulsions.

Tannic acid, a specific commercial form of tannin, is the hydrolysable tannin with high molecular weight due to its multiple phenolic groups. Tannic acid is affirmed as Generally Recognized as Safe (GRAS) by the Food and Drug Administration (FDA) for the use as a direct additive in food products. Tannin contains sufficient hydroxyls and other groups such as carboxyls to form strong complex with proteins and other macromolecules (Balange & Benjakul, 2009). Surfactant-free foamulsions with a high oil-volume foraction has been stabilized by colloidal complexes prepared by the interactions between methylcellulose (MCE) and tannic acid (Patel, Drost, Blijdenstein, & Velikov, 2012). The binding of protein to tannin is believed to be primarily due to the formation of multiple hydrogen bonds between the hydroxyl group of the tannin and the carboxyl group of the proteins (Haslam, 1974). Additionally, tannin-protein complex may also stabilized by hydrophobic interactions between the aromatic ring of tannins and hydrophobic regions of proteins (Hager, Vallons, & Arendt, 2012). It also has been reported that tannin-protein interactions are dominated by hydrophobic attraction and hydrogen binding of tannins to the tannin receptor sites, mostly proline residues, within the protein (Zanchi, Narayanan, Hagenmuller, Baron, Guyot, Cabane, et al., 2008). Hence, the complexations of tannic acid with proline-rich proteins such as salivary protein (Cala et al., 2012), gliadin from gluten (Siebert, Troukhanova, & Lynn, 1996) and kafirin from sorghum (Emmambux, Stading, & Taylor, 2004) have well been studied. Additionally, the protein-phenolic complex can concentrate at the O/W interface, yielding the surface-active nature of the protein and antioxidative activity of phenolic compounds (Almajano & Gordon, 2004). Therefore, an appropriate modification by incorporating the phenolic compounds (e.g. tannin) could be a potential mode to obtain the modified protein with the higher emulsifying property as well as antioxidant activity. However, little information on the interaction between proteins hydrolysate and TA as well as the emulsifying activity of their complex has been reported.

Algal oil is a rich source of polyunsaturated fatty acids (PUFAs), especially ω-3 and ω-6 fatty acids, which has been claimed for its health benefits (Kralovec, Zhang, Zhang, & Barrow, 2012; Ramakrishnan, Ferrando, Aceña-Muñoz, De Lamo-Castellví, & Güell, 2013). Nevertheless, rapid lipid oxidation severely limits the utilization of algal oil in processed foods or as a nutritional supplement in fortified food. Fortunately, the emulsion-based colloidal systems provide a promising solution for functional oil or lipophilic active components delivery. Therefore, the objectives of this study were to investigate the colloidal complexation ZH with TA and more importantly, to utilize the ZH-TA complex to construct a nanoemulsions system for algal oil delivery.

2. Materials and methods

2.1. Materials

Zein (>92%, dt%), tannic acid, and amino acid standards were all purchased from Sigma–Aldrich (St. Louis, MO). Alcalase 2.4 L (endoproteinase from Bacillus licheniformis, 2.4 AU/g) was obtained from Novozymes (China) biotechnology co., LTD. Algal oil was supplied by Runke bioengineering co., LTD (China). All other chemicals used were of analytical grade.

2.2. Preparation and characterization of ZH

ZH was prepared according to our previous method (Wang, Wang, Yang, Guo, & Lin, 2015) with slightly modifications. Briefly, zein aqueous suspension (3% w/v) was hydrolyzed with Alcalase at 50 °C in automatic potentiometric titrator (Metrohm). The mass ratio of enzyme to substrate was 2:100. The pH of zein solution was adjusted to 9.0 before hydrolysis was initiated, and it was maintained to 9.0 by continuing dropwise adding 1 M NaOH during hydrolysis. After 2 h hydrolysis (preliminary experiments showed that native zein can be completely liquefied after 2 h hydrolysis), the pH of the broths was brought to 7.0 using 1 M HCl, and the solution was then heated at 95 °C for 5 min to inactivate the enzymes. Then the hydrolysate was centrifuged at 10,000 r/min for 20 min at 25 °C. The supernatant was dialyzed (100 Da cutoff) over night against deionized water to desalt and finally freeze-dried (Dura-Dry MP freeze-dryer, FTS Systems, Inc., Ridge, NY). The prepared ZH was stored at 4 °C before use.

Molecular weight distributions of ZH were determined by Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI/TOF/MS) according to the method of Xu and coworkers (Xu et al., 2006) with minor modifications. ZH solution (1 mg/mL) in deionized water was filtered through a Minisart RC4 filter (0.45 μm) and mixed with 1 volume of matrix solutions (20 mg/mL of sinapinic acid in acetonitrile/water, 50:50, v/v). 0.5 μL of the mixture was deposited onto the MALDI target plate. MALDI/TOF/MS measurements were performed on a Bruker Autoflex time-flight mass spectrometer (Bruker Co., Bremen, Germany), equipped with a pulsed nitrogen laser operated at 337 nm, capable of executing a linear mode. 30 laser shots were added for the spectrum.

2.3. Interactions between ZH and TA

2.3.1. Turbidity

Stock solutions of ZH and TA were prepared by dissolving the two substances in PBs (10 mM, pH 7.0) respectively with a concentration of 10 mg/mL. PBs (used as the diluting solvent) and TA solution were respectively added into ZH solution (1.5 mL) until the final mass ratio of TA/ZH up to 0, 0.1, 0.2, 0.3, 0.5, 0.8 and 1.0. After 5 min shaking at room temperature, the absorbance of the mixture was recorded at 600 nm as the turbidity values.

2.3.2. Fluorescence titration

Intrinsic fluorescence of ZH was measured by fixing the concentration of ZH at 1 mg/mL in PBs (10 mM, pH 7.0) and varying the concentrations of TA from 0 to 10 μg/mL. Emission spectra were recorded from 290 to 400 nm at an excitation wavelength of 280 nm. Quenching of protein fluorescence due to energy transfer from the tyrosine (Tyr) residue to TA served to determine the binding of ZH with TA. Fluorescence quenching is described